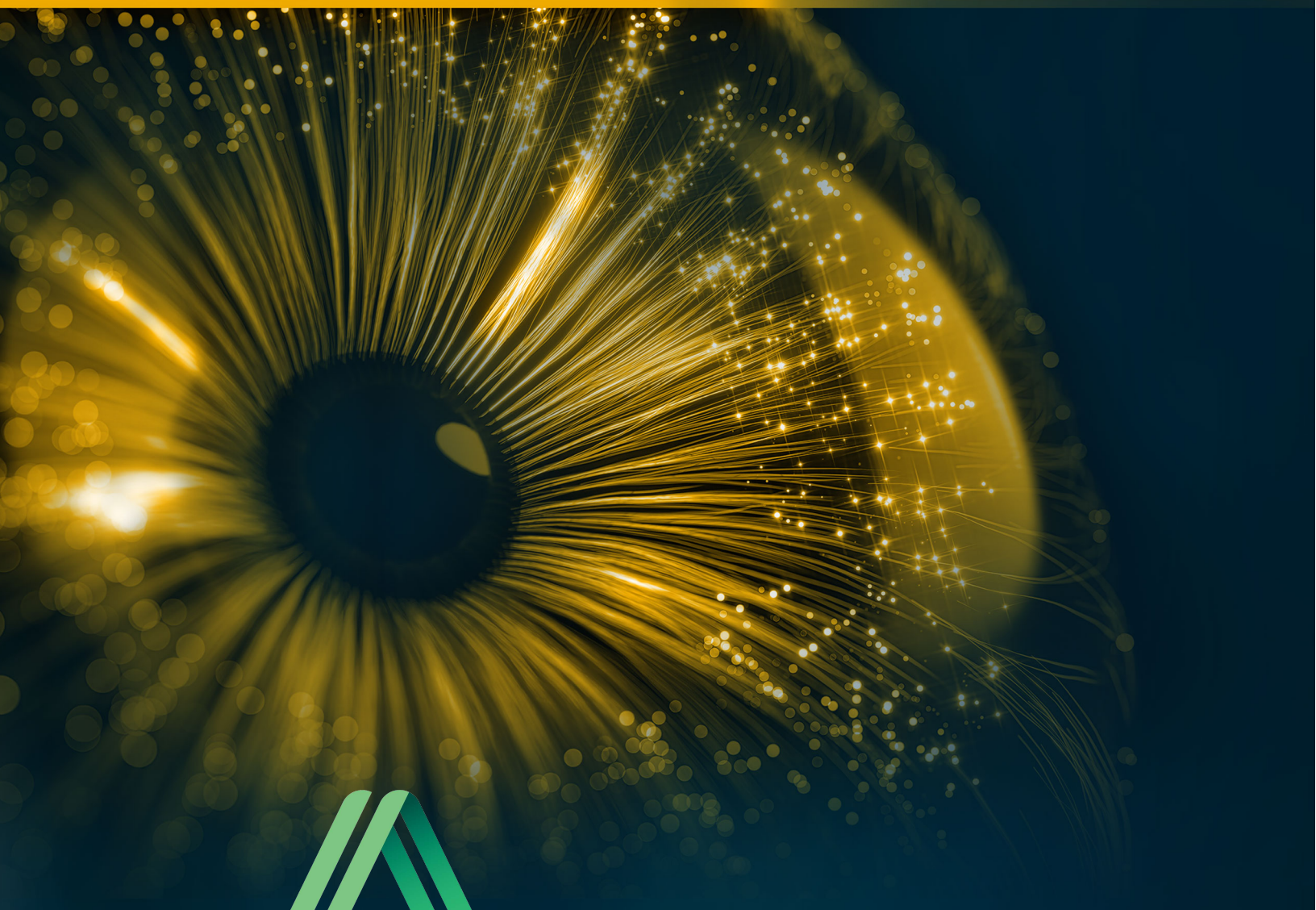
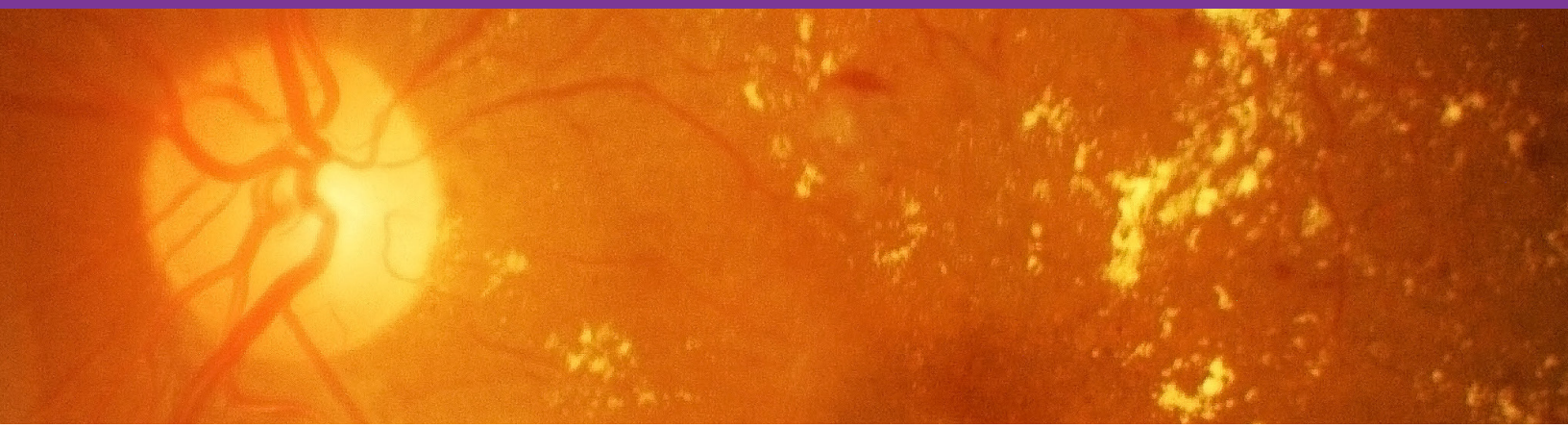


ALTASCIENCES

SAFETY ASSESSMENT FOR OPHTHALMIC PRODUCTS



PRECLINICAL RESEARCH

Preclinical trial design for ocular drugs depends on the specifics of the drug in development, such as whether the drug product is a new chemical entity (NCE), or a reformulation of a previously approved drug.

Preparation for First-in-Human (FIH) Studies

When preparing for FIH studies of ocular drugs, two non-rodent species are preferred. Large species such as dogs and rabbits are suitable for drugs delivered in all routes: topical, subconjunctival, intracameral, subretinal, and intravitreal (IVT). Pigs are more suitable for subretinal and IVT injections and nonhuman primates (NHPs) are more suitable for subconjunctival, intracameral, subretinal, and IVT. Dogs and small species like rats and mice that carry retinal mutations similar to retinitis pigmentosa (RP) are best suited for subretinal injections for gene therapy. A single species may be acceptable in cases where there is sufficient rationale (i.e., lack of biologic homology in other species), or existing nonclinical and/or clinical data from other ocular administrations of the drug.

The conduct of ocular pharmacokinetic (PK) studies, to understand the absorption, distribution, metabolism, and excretion (ADME) in various ocular compartments, is common. Usually, a single species is sufficient, and target pharmacological relevance is not required.

Before considering pivotal repeat-dose ocular studies, an ocular tolerability study is recommended; usually, a single-dose study in a small number of rabbits. Typical dosing would be a 20 to 30 µl drop size in a single dose/concentration, with hourly observation and scoring (modification of the Draize scoring system), and concentration or dose frequency increases over several days, with complete ophthalmological evaluation by slit-lamp biomicroscopy, and indirect ophthalmoscopy

For pivotal toxicity studies, typical parameters for ocular evaluation include ophthalmic examinations, including biomicroscopy of the anterior segment, indirect ophthalmoscopy to examine the posterior segment, tonometry, optical coherence tomography (OCT) or electroretinograms (ERG) if the drug reaches the retina, and histopathology. Systemic toxicokinetic (TK) analysis is typically performed, particularly when systemic tissues are being evaluated. Ocular TK is rarely performed because there is insufficient tissue for compartmentalization studies. Microscopic examination and ocular PK studies are thus acceptable. If necessary, other specialized procedures such as fluorescein angiography or vitreal PK may be performed.

Before FIH trials, a systemic evaluation of toxicity in at least one species using intravenous or oral administration to maximize systemic exposure is generally expected, as are two in vitro genetic toxicity tests for mutation and clastogenicity. A full systemic evaluation in one or both species, as well as a separate study in one species, such as a rat, can be included in the ocular development program. When necessary, reproductive toxicity and safety pharmacology should be evaluated in accordance with ICH guidelines.

Species and Strain Selection Parameters

Nonrodents such as rabbits, dogs, and/or monkeys are usually the species of choice in ocular toxicity and pharmacokinetic studies. Due to the large size of their eye, rabbits are the most commonly used species for ocular toxicity testing. A nonpigmented rabbit strain, such as the New Zealand White (NZW), is usually acceptable when combined with a pigmented species (dog or primate). Minipigs are frequently used to study human ocular disease because the retina, pupil, and lens most closely resemble those of the human eye. Dogs are good models as different breeds carry different relevant mutations, and they have large eyes that allow for higher volume injections and treatment areas.

The anatomy and physiology of the monkey eye are most similar to that of the human eye, including the presence of a macula. NHPs are typically the first species chosen for biologics due to higher sequence homology and relevance to pharmacological responses in humans, as well as a lower risk of antigenic response to the test article. Dogs and small rodent species such as rats and mice, where strains with genetic retinal mutations are easily studied, are used for subretinal gene therapy injections.

Routes of Administration

Different formulations and routes of administration allow for optimal drug delivery and absorption in the target structure of the eye.

ROUTES OF ADMINISTRATION	FORMULATIONS
Topical	Liquid, suspension, ointment
Subconjunctival	Liquid, suspension, gel
Intracameral	Liquid
Intravitreal	Liquid, suspension, gel, implants
Subretinal	Liquid, cell delivery

There are some important differences in ophthalmic preparations: a liquid formulation is quickly absorbed and releases drug rapidly but may require frequent administration. Ointment, gels, and implants are generally used for slow drug release, and require less frequent administration.

Specialized Ocular Assessments and Equipment

For subretinal injections, highly skilled scientists and specific equipment are needed. A specialized injection device with a 25-gauge needle penetrates the conjunctiva and the sclera. From there, when the tip of the needle approaches the retinal surface, a 39-gauge polyimide cannula is extended and brought into apposition with the retina for the subsequent subretinal injection of test article or adeno-associated virus (AAV). Using this procedure, closure of the conjunctiva is not required.

In vivo imaging after intravitreal and subretinal injections is performed via OCT and fundus photography. OCT is a non-invasive imaging technology that provides a cross-sectional view of the retina, the retinal-vitreal interface, and anterior ocular structures at near-cellular resolution. In fundus photography, a specialized low-power microscope is attached to a camera and examines structures such as the optic disc, retina, lens, and cornea. In preclinical studies, these technologies are used to help evaluate pharmacological or toxicological drug effects, and evaluate test article progression and possible toxicity to the retina/choroid.

PRECLINICAL CASE STUDY

Safety Assessment of Intravitreal Implants in Dutch Belted Rabbits

Study Overview

In support of their IND, Altasciences' client commissioned a chronic toxicology study to evaluate the ocular safety of a cylindrical intravitreal implant, which was loaded with an API intended for the treatment of age-related macular degeneration. Dutch belted (DB) rabbits were chosen based on (1) the historical use of this breed for ocular safety assessment, (2) the need for an animal model with pigmented eyes to evaluate the potential for ocular melanin binding, and (3) the availability of animals in sufficient quantity for an IND-enabling study.

As is often the case for nonclinical safety studies, the doses given to the rabbits needed to be several-fold higher than the human dose, which required that up to six implants be delivered to each eye. The large number of implants presented both technical and scientific challenges. Rabbits have a much smaller vitreous space (~1.5 mL total vitreous volume in rabbit versus ~5 mL in human) and larger lens (~8 mm in rabbit versus ~4 mm in human). These factors present an increased risk of the implants coming into contact with the soft tissues in the posterior segment of the eye during injection, and potentially post-injection. Special consideration was needed when designing the study to ensure that any lesions resulting from the dosing procedure could be identified, monitored throughout the study, and ultimately differentiated from API-related effects.

Study Details

<table>
<tr><td>Animal model: DB rabbits</td><td rowspan="3">General observations: clinical signs, body weights, food consumption</td></tr>
<tr><td>Duration of study: 1.5 years</td></tr>
<tr><td>No. of animals: 7M/7F per cohort</td></tr>
<tr><td>Dose route: intravitreal implantation</td><td rowspan="2">Ocular measurements/observations: ophthalmic examination, intraocular pressure, electroretinogram, fundus photography, ocular histopathology</td></tr>
<tr><td>Dose regimen: single intravitreal delivery to both eyes on Day 1</td></tr>
</table>

Study Purpose

The objective of the study was to assess the toxicity and toxicokinetics of the sponsor's intravitreal implants for a period of up to 1.5 years following a placement of the implants in the vitreous space of both eyes in DB rabbits.

Methods

Animals were dosed by intravitreal injection using proprietary injectors provided by the sponsor. All eyes were dosed once at the start of the study according to the table below.

GROUP	TREATMENT	# OF IMPLANTS PER EYE
Control	Placebo implant	2
Test article low-dose	Test implant	2
Test article mid-dose	Test implant	3
Test article mid-dose	Test implant	4
Test article high-dose	Test implant	6

All test implants contained the same mg amount of API. Implants were delivered with a single injection (control and low dose) or two successive injections (mid- and high-dose).

Standard safety observations and measurements were performed over the course of the study, including detailed clinical observations, body weights, food consumption, and clinical pathology.

Ocular assessments were done prior to injection, once during the week following injection, approximately once a month during the first year, and then approximately once every two months during the final six months of the study.

Ocular assessments included fundus imaging (RetCam Shuttle), intraocular pressure (Reichert TonoPen), ophthalmology examination, and electroretinography. Ophthalmology examinations were performed by a board-certified veterinary ophthalmologist, and included examination of the anterior segment with the aid of a slit-lamp and examination of the fundus using an indirect ophthalmoscope and an aspheric condensing lens.

Blood samples were collected periodically over the course of 18 months to assess systemic exposure.

Animals were euthanized at approximately six months and 18 months following placement of the implants. Complete necropsies were conducted, and standard organ weights were recorded. The eyes were collected and preserved en bloc for histopathology evaluation. The eyes were then sectioned at three levels, processed to slide, and stained with hematoxylin and eosin. Slides were read by a board-certified veterinary pathologist.

Results

Although minimal systemic exposure to the API was detected in test group animals, there was no evidence of systemic effects.

Non-adverse findings were noted for the eyes during the in-life phase.

The pathologist noted microscopic findings in the vitreous, lens and retina of control, and test eyes. All vitreous findings were deemed non-adverse due to limited severity. Microscopic observations noted for the lens were considered injection procedure-related and adverse. Microscopic findings in the retina were considered to be injection procedure-related, some adverse and some not.

Electroretinography revealed waveform abnormalities in some eyes that received six implants, including reduced b-wave positivity in the dark-adapted response reduced light-adapted response.

The general appearance of the implants was observed by indirect ophthalmoscopy and fundus imaging. The implants remained intact over the course of the study. A gradual decrease in coloration of the API-containing implants was observed and was attributed to depletion of the test drug.

Conclusion

The technical and scientific challenges associated with the placement of multiple implants in the relatively small vitreous space of DB rabbits required specialized instruments and qualified experts in the fields of veterinary ophthalmology, veterinary pathology, and electroretinography. The combined expertise of our team, familiarity with the test species, and commitment to making the review and interpretation of the data a collaborative effort, allowed for us to differentiate between effects associated with the injection procedure, the API, and/or the implant itself. These distinctions can be critical to acceptance of the study results by regulatory agencies.

ALTASCIENCES' RESOURCES

Fact Sheet

Ophthalmology

Webinars/Podcasts

Nonclinical Considerations when Developing an Ophthalmic Drug

Terminal Sterilization

Development of Nanosuspension Formulations for Poorly Soluble Drugs

INTEGRATED SOLUTION

Webpages

Ophthalmic End-to-End Drug Development Solutions

Proactive Drug Development

A.T.L.A.S. Customized End-to-End Approach

Scientific Poster

In Vivo and Historical Analysis of Focal Chorioretinal Defects in Dutch Belted Rabbits

ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements. Altasciences helps sponsors get better drugs to the people who need them, faster.